学　位　論　文　要　旨

Heat shock induced resistance in tomato - molecular mechanism and utilization in practical field -
トマトにおける熱ショック誘導抵抗性—分子機構と実用—

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Due to their sessile nature, plants are often exposed to environmental stress. The acquisition of higher levels of stress tolerance is of utmost importance to plants for survival. Unlike any other stress condition, plants under heat stress must act quickly to survive. However, this fast response to heat stress leads to the acquisition of tolerance against many other stressful conditions, such as water deficiency, high salt, chemical pollutants, oxidative stress, nematodes, herbivores, extreme temperatures, and pathogens. Known as “heat shock-induced resistance” (HSIR), short and acute exposure of plants to hot water induces plant resistance against pathogens. In the present study, I focused on the molecular mechanism of HSIR by studying the regulation of the heat stress response by heat shock transcription factors (Hsfs). In addition, I also examined the possibility for a practical application of heat shock treatment (HST) to induce pathogen resistance in tomatoes. To gain insight into the mechanism of regulation, I used tomato seedlings, for which the genome was fully sequenced and well annotated. I investigated the role of Hsfs during induction of defense response by HST.

Leaf disease symptoms were significantly reduced at 12 and 24 h after HST, consistent with the upregulation of pathogenesis-related (PR) genes *PR1a2* and *PR1b1* peaking 24 h after treatment. These genes were upregulated at the treatment application site, but not in untreated leaves. In contrast to HST, inoculation of the first leaf induced the systemic upregulation of acidic PR genes in uninoculated second leaves. Furthermore, heat shock element motifs were found in upstream regions of *PR1a2*, *PR1b1*, *Chitinase 3* (*Chi3*), *Chitinase 9* (*Chi9*), *Glucanase A* (*GluA*), and *Glucanase B* (*GluB*) genes. The relative expression of *HsfA2* and *HsfB1* peaked at 6 h after HST, which was 6 h earlier than the time when salicylic acid accumulation was observed. Foliar spray of heat shock protein 90 (Hsp90) inhibitor geldanamycin

(GDA) induced both acidic (*PR1a2*, *Chi3*, and *GluA*) and basic (*PR1b1*, *Chi9*, and *GluB*) PR gene expression, comparable to HST. PR gene expression and defense response against *Pseudomonas syringae* pv. *tomato* (*Pst*) decreased when combining HST with Hsfs inhibitor KRIBB11. The Hsfs and PR gene expression induced by heat or GDA, together with the suppression of HSIR against *Pst* by KRIBB11, suggested a direct contribution of Hsfs to HSIR regulation in tomato plants.

Practical field application of HST for inducing plant resistance against pathogens was tested using an improved hot water sprayer device against powdery mildew in a tomato nursery. In plant nurseries, reducing the frequency of chemical application is becoming a challenge owing to the appearance of hard to control pathogens, spread of diseases, and demand by farmers. This study was therefore conducted to develop a practical alternative fungal control strategy against powdery mildew by using a hot water sprayer in a tomato nursery. The expected effects of the hot water spray treatment were to induce resistance and disinfection. Gray mold was used as an experimental model to determine the conditions for a practical application of the hot water spray for inducing resistance to plant fungi by heat shock treatment. Hot water dipping of tomato seedlings at 50 °C for 20 s induced resistance against gray mold and increased the expression of some PR genes, viz., pathogenesis-related protein 1a (PR1a), GluB, and Chi9. A prototype of a towable hot water sprayer was developed, and its performance was tested in the field. This sprayer was rolled on a rail, using an electric winch installed at the end of the nursery bench. A temperature higher than 50 °C for 20 s is required to attain the optimum conditions, because of heat loss due to vaporization. Moreover, heating time must be a 20 s duration at the target leaf. In other words, at least one part of the seedling must fall under the moving spray area of hot water during hot water spray treatment (HWS) + 20 s.

The severity of powdery mildew in HWS was significantly lower than that in control seedlings. The results of tomato HWS confirmed that partial achievement of optimum conditions in the whole plant succeeded in preventing powdery mildew. The possibility that Hsfs function as triggering molecules in HSIR provides new insights into the molecular mechanisms of plant defense systems against pathogens, as well as the opportunity to develop new approaches for crop protection. Further, if Hsfs were also induced by infection, they can be proposed as a universal trigger for the activation of a defense response. Application of HSIR by hot water spraying is suggested as an effective technique for inducing resistance against powdery mildew in tomato nurseries and reducing the frequency of chemical application.